

Supplemental Experimental Procedure:

Identification and assignment of criteria for enriched candidate proteins:

The procedure used to analyze the elution pools from the *in vivo* pull down experiment is similar to as reported previously (Bhat et al., 2013). Briefly, a 'short-gel' protocol was followed where protein samples were electrophoresed into a 10% SDS-PAGE gel briefly to separate non-protein components. The bands were excised, digested with trypsin and analyzed by LC-MS/MS (Proteomics and Mass Spectrometry Facility; University of Massachusetts, Worcester). Mascot algorithm was used to identify proteins from peptide sequences obtained from MS/MS samples. The final spectrums were analyzed using Scaffold (Proteome software, Portland, OR). The parameters used to determine relative protein abundance were >95% protein identification, >90% peptide identification and identification of a minimum of 5 peptides per candidate protein. Further stringent criteria was applied by considering only those candidate proteins that contain at least 2 normalized total spectra from the elution pool of full length RcdA or RcdA Δ C, at least two-fold enrichment of normalized total spectra from the elution pool of full length RcdA or RcdA Δ C over mock elution pool, and at least 1.2-fold enrichment of normalized total spectra from the elution pool of RcdA Δ C over RcdA. The list of candidate proteins passing these criteria is shown in Table S1.

Supplemental Tables:

Table S1. Candidate substrates identified from RcdA pull down (related to Figure 5)

CC_number	CCNA_number	Candidate protein	Normalized total spectra			ratio
			M2	M2-RcdA	M2-RcdA Δ C	
CC_3315	CCNA_03424	AAA+ family response regulator-TacA	7.1	41.6	85.2	2.0
CC_3144	CCNA_03246	hypothetical protein-CCNA_03246	0.0	14.2	24.3	1.7
CC_2323	CCNA_02408	hypothetical protein-CCNA_02408	5.3	15.8	24.3	1.5
CC_1652	CCNA_01724	hypothetical protein CCNA_01724	0.0	3.3	5.7	1.7
CC_0740	CCNA_00777	PAS family GGDEF/EAL protein	0.0	2.5	14.6	5.8
CC_2764	CCNA_02852	hypothetical protein-CCNA_02852	0.0	9.2	13.0	1.4
CC_2362	CCNA_02447	alpha helical protein	0.0	3.3	4.1	1.2
CC_0752	CCNA_00789	transcriptional regulator FixK	0.0	3.3	8.1	2.4
CC_3047	CCNA_03142	RNA polymerase sigma factor-RpoD	5.3	11.7	13.8	1.2
CC_0806	CCNA_00849	outer membrane efflux protein	0.0	4.2	13.0	3.1
CC_2928	CCNA_03023	TonB-dependent receptor	1.8	4.2	13.0	3.1
CC_1984	CCNA_02063	YfiO family outer membrane assembly lipoprotein-BamD	0.0	2.5	6.5	2.6
CC_1915	CCNA_01992	outer membrane protein assembly factor-BamA	1.8	7.5	18.7	2.5
CC_0808	CCNA_00851	periplasmic multidrug efflux lipoprotein	1.8	4.2	9.7	2.3

CC_1015	CCNA_01067	type I secretion outer membrane protein-RsaF	1.8	5.0	11.4	2.3
CC_1653	CCNA_01725	YfgL-family outer membrane assembly lipoprotein-BamB	1.8	5.0	9.7	1.9
CC_0925	CCNA_00974	Cluster of OAR protein	5.3	11.7	17.9	1.5
CC_2092	CCNA_02174	multidrug resistance efflux pump	1.8	5.0	6.5	1.3
CC_1008	CCNA_01060	type I protein secretion ATP-binding protein-RsaD	0.0	2.5	7.3	2.9
CC_1009	CCNA_01061	type I secretion adaptor protein-RsaE	1.8	9.2	13.0	1.4
CC_1062	CCNA_01115	two component sensor histidine kinase	0.0	5.0	6.5	1.3
CC_2935	CCNA_03030	cytochrome c	1.8	5.0	6.5	1.3
CC_0593	CCNA_00629	methyl-accepting chemotaxis protein	0.0	4.2	8.1	1.9
CC_0430	CCNA_00439	methyl-accepting chemotaxis protein	0.0	7.5	11.4	1.5
CC_2995	CCNA_03090	acetyl-CoA carboxylase carboxyltransferase subunit alpha	0.0	3.3	6.5	1.9
CC_3176	CCNA_03280	indolepyruvate ferredoxin oxidoreductase	0.0	9.2	13.8	1.5
CC_3604	CCNA_03718	NADH-ubiquinone oxidoreductase subunit	1.8	5.0	7.3	1.5
CC_0374	CCNA_00378	thiol:disulfide interchange protein DsbA	1.8	4.2	5.7	1.4
CC_0088	CCNA_00086	NAD-specific glutamate dehydrogenase-GdhZ	5.3	11.7	14.6	1.3
CC_3521	CCNA_03636	biotin synthase	0.0	3.3	4.1	1.2
CC_1334	CCNA_01395	electron transfer flavoprotein-ubiquinone oxidoreductase	0.0	4.2	4.9	1.2
CC_2638	CCNA_02721	M16 family peptidase-PqqL	0.0	3.3	15.4	4.6
CC_3226	CCNA_03334	cell division protein FtsH	1.8	7.5	8.9	1.2

Table S2. Strains and plasmids used in this study (related to experimental procedures).

Organism	Name	Description	Source
<i>E. coli</i>	TOP10	cloning strain	Invitrogen
	BL21(DE3)	recombinant protein expression strain	Invitrogen
<i>C. crescentus</i>	CB15N	synchronizable derivative of wild-type CB15	Evinger and Agabian, 1977
	CPC133	CB15N <i>tacA</i> ::pENTR- <i>tacA</i> (<i>kan</i> ^R)	Bhat et al., 2013
	CPC134	CB15N <i>tacA</i> ::pENTR- <i>tacADD</i> (<i>kan</i> ^R)	Bhat et al., 2013
	CPC165	CB15N <i>ΔcpdR</i> (<i>tet</i> ^R)	Skerker et al., 2005
	CPC164	CB15N <i>ΔtacA</i> (<i>tet</i> ^R)	Skerker et al., 2005
	CPC239	CB15N <i>ΔpopA</i> (<i>tet</i> ^R)	K. Ryan (UC Berkeley)
	CPC452	CB15N <i>ΔrcdA</i> (<i>hyg</i> ^R)	McGrath et al., 2006
	CPC250	CB15N <i>ΔrcdA</i> <i>xyI/X</i> :: <i>rcdAΔC</i>	Taylor et al., 2009
	CPC230	CB15N <i>WT</i> :: pHXM - <i>P_{xyI/X}</i> - <i>tacA</i> (<i>spec</i> ^R)	This study
	CPC251	CB15N <i>ΔpopA</i> :: pHXM - <i>P_{xyI/X}</i> - <i>tacA</i> (<i>spec</i> ^R)	This study
	CPC283	CB15N <i>ΔrcdA</i> :: pHXM - <i>P_{xyI/X}</i> - <i>rcdAΔC</i> (<i>spec</i> ^R)	This study
	CPC295	CB15N <i>WT</i> :: pLXM- <i>P_{xyI/X}</i> -CC2323 (<i>tet</i> ^R)	This study
	CPC297	CB15N <i>ΔrcdA</i> :: pLXM- <i>P_{xyI/X}</i> - <i>rcdA</i> (<i>kan</i> ^R)	This study

	CPC301	CB15N $\Delta rcdA$:: pHXM - P_{xyIX} - $rcdA$ ($spec^R$)	This study
	CPC303	CB15N $\Delta rcdA$:: pHXM - P_{xyIX} -empty vector ($spec^R$)	This study
	CPC317	CB15N $\Delta cpdR$:: pHXM - P_{xyIX} - $rcdA$ ($spec^R$)	This study
	CPC353	CB15N $\Delta cpdR$:: pHXM - P_{xyIX} - $rcdA\Delta C\sim XB$ ($spec^R$)	This study
	CPC372	CB15N WT :: pHXM - P_{xyIX} -empty vector ($spec^R$)	This study
Expression vectors	pET23b	T7 promoter expression plasmid, (amp^R)	Invitrogen
	pET23bClpX	pET23b with <i>C. crescentus</i> <i>clpX</i>	Abel et. al., 2011
	pET23b ΔN ClpX	pET23b with <i>C. crescentus</i> <i>clpX</i> lacking <i>N-terminal domain</i>	Chien et al., 2007
	pQE70ClpP	pQE70 with <i>C. crescentus</i> <i>clpP</i> with C-terminal his ₆ (amp^R)	Chien et al., 2007
	pSUMO	pET23-hisSUMO (T7 promoter driven vector for his ₆ -SUMO fused recombinant protein expression) (amp^R)	Wang et al., 2007
	pSUMO- <i>cpdR</i>	pET23-hisSUMO with <i>cpdR</i>	Lau et al., 2015
	pSUMO- <i>pdeA</i>	pET23-hisSUMO with <i>pdeA</i>	Lau et al., 2015
	pSUMO- <i>tacA</i>	pET23-hisSUMO with <i>tacA</i>	Bhat et al., 2013
	pSUMO- <i>tacA</i>	pET23-hisSUMO with <i>tacA-DD</i>	Bhat et al., 2013
	pSUMO- <i>tacA</i> (2-116)	pET23-hisSUMO with <i>tacA</i> containing residues from 2-116	This work
	pSUMO- <i>tacA</i> (117-488)	pET23-hisSUMO with <i>tacA</i> containing residues from 117-488	This work
	pSUMO- <i>tacA</i> (312-488)	pET23-hisSUMO with <i>tacA</i> containing residues from 312-488	This work
	pSUMO- <i>tacA</i> (437-488)	pET23-hisSUMO with <i>tacA</i> containing residues from 437-488	This work
	pSUMO- <i>tacA</i> (449-488)	pET23-hisSUMO with <i>tacA</i> containing residues from 449-488	This work
	pSUMO-CC2323	pET23-hisSUMO with CC2323	This work
	pHISDEST	T7 promoter expression plasmid, N-terminal his ₆ tag, Destination vector (amp^R)	Skerker et. al., 2005
	pHIS- <i>tacA</i>	pHISDEST with <i>tacA</i>	This study
	pHIS- <i>ctrA</i>	pHISDEST with <i>CtrA</i>	Chien et al., 2007
	pBAD-GFP-ssrA	pBAD with GFP-ssrA	Lau et al., 2015
	pHIS-GFP-ssrASS	pHISDEST with GFP-ssrASS	Lau et al., 2015
	pET28a or pET28b	T7 promoter expression plasmid, N-terminus his tag, thrombin cleavable, (kan^R), a and b varies in MCS	Novagen
	pET28a-his ₆ -RcdA	pET28a with <i>rcdA</i>	Chien et al., 2007
	pET28a- his ₆ -RcdA ΔC	pET28a with <i>rcdAΔC</i>	K. Ryan (UC Berkeley)
	pET28a-his ₆ -RcdA-L160D	pET28a with <i>rcdA-L160D</i>	This study
	pET28a-his ₆ -RcdA-L163D	pET28a with <i>rcdA-L163D</i>	This study

	pET28a-his ₆ -RcdA-F167D	pET28a with <i>rcdA-L167D</i>	This study
	pET28a-his ₆ -RcdA-D161A,R162A	pET28a with <i>rcdA-D161A,R162A</i>	This study
	pET28a-his ₆ -PopA	pET28a with <i>popA</i>	This work
	pET28b-his ₆ -SspB	pET28b with <i>sspB</i>	Chien et al., 2007
	pET28b-his ₆ -SspB-125	pET28b with <i>sspB-125</i>	Chien et al., 2007
	pET28b-his ₆ -SspB~RcdA chimera	pET28b with <i>sspB</i> lacking C-terminal 10 residues and appended with C-terminal 19 residues of RcdA	This study
	pET28b-his ₆ -RcdAΔC~XB	pET28b with <i>rcdA</i> lacking 19 residues from C-terminus and appended with C-terminus 10 residues of SspB	This study
	pET28a-his ₆ -CC3144	pET28b with CC3144	This study
	pHXM-DEST	pJS71-PxyIX-M2; high-copy, xylose inducible, N-terminus M2 tag (<i>spec^R</i>)	Skerker, et al. 2005
	pLXM-DEST(kan)	pMR10-PxyIX-M2; broad host range, low-copy, xylose inducible, N-terminus M2 tag (<i>kan^R</i>)	Bhat, et al. 2013
	pLXM-DEST(tet)	pMR20-PxyIX-M2; broad host range, low-copy, xylose inducible, N-terminus M2 tag (<i>tet^R</i>)	Skerker, et al. 2005
Entry vectors	pENTR/D-TOPO	ENTRY vector for Gateway cloning (<i>kan^R</i>)	Invitrogen
	pENTR- <i>rcdA</i>	<i>rcdA</i> in pENTR/D-TOPO (<i>kan^R</i>)	This work
	pENTR- <i>rcdA</i> ΔC	<i>rcdA</i> ΔC in pENTR/D-TOPO (<i>kan^R</i>)	This work
	pENTR- <i>rcdA</i> ΔC~XB	<i>rcdA</i> ΔC~XB in pENTR/D-TOPO (<i>kan^R</i>)	This work
	pENTR-CC2323	CC2323 in pENTR/D-TOPO (<i>kan^R</i>)	This work

Supplemental References:

1. Abel, S., Chien, P., Wassmann, P., Schirmer, T., Kaever, V., Laub, M. T., Baker, T. A., and Jenal, U. (2011). Regulatory cohesion of cell cycle and cell differentiation through interlinked phosphorylation and second messenger networks. Mol. Cell 43, 550-560.
2. Bhat, N. H., Vass, R. H., Stoddard, P. R., Shin, D. K., and Chien, P. (2013). Identification of ClpP substrates in *Caulobacter crescentus* reveals a role for regulated proteolysis in bacterial development. Mol. Microbiol. 88, 1083-1092.
3. Chien, P., Perchuk, B. S., Laub, M. T., Sauer, R. T., and Baker, T. A. (2007). Direct and adaptor-mediated substrate recognition by an essential AAA+ protease. Proc. Natl. Acad. Sci. 104, 6590-6595.
4. Evinger, M. and Agabian, N. (1977). Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J. Bacteriol. 132, 294-301.
5. Lau, J., Hernandez-Alicea, L., Vass, R. H., and Chien, P. (2015). A phosphosignaling adaptor primes the AAA+ protease ClpXP to drive cell cycle regulated proteolysis. Mol. Cell 59, 104-116.

6. McGrath, P. T., Iniesta, A. A., Ryan, K. R., Shapiro, L., and McAdams, H. H. (2006). A dynamically localized protease complex and a polar specificity factor control a cell cycle master regulator. *Cell* 124, 535-547.
7. Skerker, J.M., Prasol, M.S., Perchuk, B.S., Biondi, E.G. and Laub, M.T. (2005). Two-component signal transduction pathways regulating growth and cell cycle progression in a bacterium: a system-level analysis. *PLoS Biol.* 3, 1770-1788.
8. Taylor, J., Wilbur, J. D., Smith, S. C., and Ryan, K. R. (2009). Mutations that alter RcdA surface residues decouple protein localization and CtrA proteolysis in *Caulobacter crescentus*. *J. Mol. Biol.* 394, 46-60.
9. Wang, K.H., Sauer, R.T., Baker, T.A. (2007). ClpS modulates but is not essential for bacterial N-end rule degradation. *Genes Dev.* 21, 403-408.